



# Histamine H<sub>1</sub>-Receptors Modulate Somatostatin Receptors Coupled to the Inhibition of Adenylyl Cyclase in the Rat Frontoparietal Cortex

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PUEBLA, L., A. OCAÑA AND E. ARILLA. *Histamine H<sub>1</sub>-receptors modulate somatostatin receptors coupled to the inhibition of adenylyl cyclase in the rat frontoparietal cortex*. *Peptides* **18**(10) 1569–1576, 1997.—Since exogenous histamine has been previously shown to increase the somatostatin (SS) receptor-effector system in the rat frontoparietal cortex and both histamine H<sub>1</sub>-receptor agonists and SS modulate higher nervous activity and have anticonvulsive properties, it was of interest to determine the participation of the H<sub>1</sub>-histaminergic system in this response. The intracerebroventricular (i.c.v.) administration of the specific histamine H<sub>1</sub>-receptor agonist 2-pyridylethylamine (PEA) (10 µg) to rats 2 h before decapitation increased the number of SS receptors (599 ± 40 vs 401 ± 31 femtomoles/mg protein,  $p < 0.01$ ) and decreased their apparent affinity for SS (0.41 ± 0.03 vs 0.26 ± 0.02 nM,  $p < 0.01$ ) in rat frontoparietal cortical membranes. No significant differences were seen for the basal and forskolin (FK)-stimulated adenylyl cyclase (AC) activities in the frontoparietal cortex of PEA-treated rats when compared to the control group. In the PEA group, however, the capacity of SS (10<sup>-4</sup> M) to inhibit basal and FK (10<sup>-5</sup> M)-stimulated AC activity in frontoparietal cortical membranes was significantly higher than in the control group (34 ± 1% vs 20 ± 2%,  $p < 0.001$ ). The ability of low concentrations of the stable GTP analogue 5'-guanylylimidodiphosphate [Gpp(NH)p] to inhibit FK-stimulated AC activity in frontoparietal cortical membranes was similar in the PEA-treated and control animals. These results suggest that the increased SS-mediated inhibition of AC activity in the frontoparietal cortex of PEA-treated rats may be due to the increase of the number of SS receptors induced by PEA. Pretreatment with the H<sub>1</sub>-receptor antagonist mepyramine (30 mg/kg, intraperitoneally (IP)) prevented the PEA-induced changes in SS binding and SS-mediated inhibition of AC activity. Mepyramine (30 mg/kg, IP) alone had no observable effect on the somatostatinergic system. The *in vitro* addition of PEA or mepyramine to frontoparietal cortical membranes obtained from untreated rats did not affect the SS binding parameters. Altogether, these results suggest that the H<sub>1</sub>-histaminergic system modulates the somatostatinergic system in the rat frontoparietal cortex. © 1997 Elsevier Science Inc.

Mepyramine      2-Pyridylethylamine      Somatostatin receptor      Adenylyl cyclase      Rat      Frontoparietal cortex

SEVERAL immunocytochemical studies have demonstrated the existence of histaminergic neurons in the brain which are concentrated in the tuberomammillary nucleus of the posterior hypothalamus and which project efferent fibers to almost all parts of the brain, including the cerebral cortex (45,63). Histamine has been shown to act as a neuroregulator or neurotransmitter in the mammalian central nervous system (CNS) (49,67) and has been implicated in the regulation of higher nervous activity (19,43) and behavioral arousal (33,64) through its interaction with H<sub>1</sub>-receptors (54). Somatostatin (SS), a tetradecapeptide widely distributed throughout the CNS (26), regulates many aspects of CNS function including arousal (17) and higher nervous activity (26) and may act as an endogenous anticonvulsant (35,48). In addition, it has been shown that SS decreases cortical histamine levels (9,10). We recently demonstrated that exogenous histamine increases the SS receptor-

effector system in the rat frontoparietal cortex (50). It is not unreasonable to suggest, therefore, that the H<sub>1</sub>-histaminergic system may modulate the somatostatinergic system. The frontoparietal cortex contains high levels of SS and H<sub>1</sub>-histaminergic receptors (24,25,44,58,60,62). The SS receptors are coupled to the adenylyl cyclase (AC) enzyme system via the guanine nucleotide-binding inhibitory protein Gi (34,40,52,55). An H<sub>1</sub>-histamine receptor agonist, 2-pyridylethylamine (PEA) (22,65), and antagonist, mepyramine (22,65), were therefore used to examine the role of the H<sub>1</sub>-histamine receptors in the regulation of the SS receptor-effector system in rat frontoparietal cortical membranes. Low concentrations of the stable GTP analogue 5'-guanylylimidodiphosphate [Gpp(NH)p] were employed to detect functional Gi-protein activity by inhibiting AC activity that has been amplified by FK (61). We extended our analysis

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in order to study the possible effects of PEA and/or mepyramine on somatostatin-like immunoreactivity (SSLI) content in the frontoparietal cortex.

#### METHOD

##### Materials

Synthetic Tyr<sup>11</sup>-SS and SS-14 were purchased from Universal Biologicals Ltd (Cambridge, UK); carrier-free Na<sup>125</sup>I (IMS 30, 100 mCi/ml) was purchased from the Radiochemical Center (Amersham, U.K.); bacitracin, bovine serum albumin (BSA), FK, mepyramine maleate, phenylmethylsulphonyl fluoride (PMSF), GTP, and 3-isobutyl-1-methylxanthine (IBMX) were purchased from Sigma (Madrid, Spain); 2-pyridylethylamine dihydrochloride (PEA) was supplied by Smith, Kline and French. The rabbit antibody used in the radioimmunoassay technique was purchased from the Radiochemical Centre (Amersham, U.K.). This antiserum was raised in rabbits against SS-14 conjugated to BSA and is specific for SS, but since SS-14 constitutes the C-terminal portions of both SS-25 and SS-28, the antiserum does not distinguish between these three forms. The binding of SS-14 to this antibody does not depend on an intact disulfide bond in the molecule as breaking of the disulfide bond by reaction with 0.1% mercaptoethanol (boiling water bath, 5 min) did not change the immunoreactivity of the peptide. Cross-reactivity with other peptides was less than 0.5%. Cross-reaction with several somatostatin analogues demonstrated that neither the N-terminal glycine nor the C-terminal cysteine residue is required for antibody binding, suggesting that the antigen site is directed towards the central part of the molecule containing the tryptophan residue.

##### Experimental Animals

The animals used in this study were sixty Wistar rats weighing between 200–250 g. Rats were maintained on a 12 h light/dark cycle (07.00–19.00) and allowed free access to food and water. Mepyramine (5, 10, 30 or 60 mg/kg) was dissolved in 0.9% NaCl and injected intraperitoneally (IP) as previously described (42). PEA (2.5, 5, 10 or 20 µg/rat) was dissolved in 0.9% NaCl (11) and injected intracerebroventricularly (i.c.v.) in a volume of 10 µl according to the method described by Noble et al. (41). In one experimental group, mepyramine (30 mg/kg, IP) was administered 1 h before PEA (10 µg, i.c.v.). Control animals for each experimental group were injected with equivalent volumes of saline. The animals were decapitated 2 h after injection of the drug. The brains were rapidly removed and the frontoparietal cortex was dissected over ice according to the method of Glowinski and Iversen (29).

##### Tissue Extraction and SS Radioimmunoassay

For SSLI measurements, the frontoparietal cortex was rapidly homogenized in 1 ml of 2M acetic acid using a Brinkman polytron (setting 5, 30 s). The extracts were boiled for 5 min in a water bath, chilled in ice, and aliquots (100 µl) were removed for protein determination (46). The homogenates were subsequently centrifuged at 15,000 × g for 15 min at 4°C and the supernatant was neutralized with 2 M NaOH. The extracts were then stored at –70°C until assay. The SSLI content was determined in tissue extracts by a modified radioimmunoassay method (46), with a sensitivity limit of 10 pg/ml. Incubation tubes prepared in duplicate contained 100 µl samples of unknown or standard solutions of 0–500 pg cyclic SS tetradecapeptide diluted in phosphate buffer (0.05 M, pH 7.2 containing 0.3% BSA, 0.01 M EDTA), 200 µl of appropriately diluted anti-SS serum, 100 µl of freshly prepared <sup>125</sup>I-Tyr<sup>11</sup>-SS diluted in buffer to give 6,000 cpm/assay tube

TABLE 1  
EFFECT OF 2-PYRIDYLETHYLAMINE (PEA), MEPYRAMINE AND MEPYRAMINE PLUS PEA ON SOMATOSTATIN-LIKE IMMUNOREACTIVE (SSLI) CONTENT AND EQUILIBRIUM PARAMETERS FOR SOMATOSTATIN (SS) BINDING IN RAT FRONTOPARIETAL CORTEX

Groups	SS receptors		SSLI
	Bmax	Kd	
Control	409 ± 30	0.27 ± 0.02	9.15 ± 0.73
PEA			
2.5 µg/rat	489 ± 18*	0.33 ± 0.03	
5.0 µg/rat	523 ± 13**	0.37 ± 0.04*	
10.0 µg/rat	603 ± 24**	0.42 ± 0.04**	7.84 ± 1.64
20.0 µg/rat	631 ± 27**	0.46 ± 0.05**	
Control	407 ± 26	0.28 ± 0.03	11.92 ± 0.99
Mepyramine			
5 mg/Kg	392 ± 16	0.25 ± 0.02	
10 mg/Kg	395 ± 14	0.24 ± 0.02	
30 mg/Kg	401 ± 19	0.24 ± 0.03	10.79 ± 0.84
60 mg/Kg	398 ± 20	0.25 ± 0.02	
Control	407 ± 26	0.28 ± 0.03	10.72 ± 1.40
Mepyramine plus PEA	381 ± 15	0.33 ± 0.03	10.38 ± 0.70

Binding parameters were calculated from Scatchard plots by linear regression. Units for SSLI are ng of SS per mg of protein, units for Kd are nM and units for Bmax are femtomoles of SS bound per mg of protein. The results are the means ± SEM of five separate experiments performed in duplicate. Statistical comparison versus control: \*  $p < 0.05$ ; \*\*  $p < 0.01$ .

(equivalent to 5–10 pg), and enough buffer to give a final volume of 0.8 ml. All reagents as well as the assay tubes were kept chilled in ice before their incubation for 48 h at 4°C. Separation of bound and free hormone was accomplished by the addition of 1 ml dextran-coated charcoal (dextran T-70: 0.2% w/v, Pharmacia, Uppsala, Sweden; charcoal Norit A: 2% w/v, Serva, Feinbiochemica, Heidelberg, Germany). Serial dilution curves for the samples were parallel to the standard curve. The intra- and inter-assay variation coefficients were 6.2 and 8.6%, respectively.

##### Binding Assay

Tyr<sup>11</sup>-SS was radioiodinated by the chloramine-T method (30). The tracer was purified in a Sephadex G-25 fine column (1 × 100 cm) which had been equilibrated with 0.1 M acetic acid containing BSA 0.1% (w/v). The specific activity of the purified labelled peptide was determined by the method of Singh et al. (57) and was approximately 600 Ci/mmol.

Membranes from rat frontoparietal cortex were prepared as previously described by Reubi (51). Membrane protein was determined by the method of Lowry (36) using BSA as a standard. Specific SS binding was measured according to the method of Czernik and Petrack (16) modified from Srikant and Patel (58). Briefly, brain membranes (0.15 mg protein/ml) were incubated in 250 µl of a medium containing 50 mM Tris-HCl buffer (pH 7.5), 5 mM MgCl<sub>2</sub>, 0.2% (w/v) BSA and 0.1 mg/ml bacitracin with 250 pM <sup>125</sup>I-Tyr<sup>11</sup>-SS either in the absence or presence of 0.01–10 nM unlabelled SS. After incubation for 60 min at 30°C, the free radioligand was separated from the bound radioligand by centrifugation at 11,000 × g for 2 min and the resultant pellet was counted in a Kontron gamma counter. Nonspecific binding was obtained from the amount of radioactivity bound in the presence of 10<sup>–7</sup> M unlabelled SS and represented about 20% of the binding

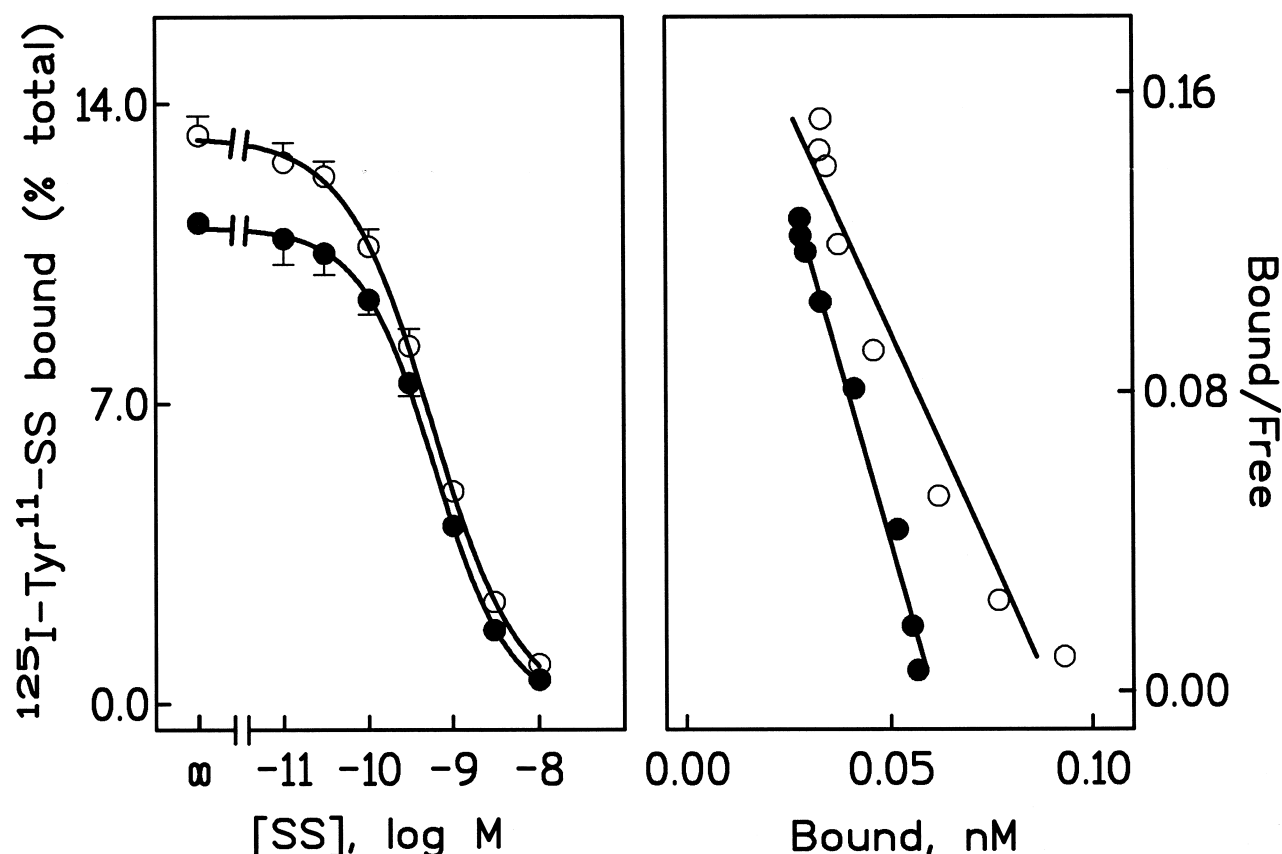


FIG. 1. Effect of 2-pyridylethylamine (PEA) (10  $\mu$ g/rat, i.c.v.) on somatostatin (SS) binding to rat frontoparietal cortical membranes. *Left panel:* Competitive inhibition of specific <sup>125</sup>I-Tyr<sup>11</sup>-SS binding by unlabelled SS to frontoparietal cortical membranes. Membranes (0.15 mg protein/ml) were incubated for 60 min at 30°C in the presence of 250 pM <sup>125</sup>I-Tyr<sup>11</sup>-SS and increasing concentrations of native peptide. Points correspond to values for the animals in the control group (●) and PEA-treated group (○). Each point is the mean  $\pm$  SEM of five replicate experiments. *Right panel:* Scatchard analysis of the binding data.

observed in the absence of unlabelled peptide. This non-specific component was subtracted from the total bound radioactivity in order to obtain the corresponding specific binding.

#### Evaluation of Radiolabelled Peptide Degradation

To determine the extent of tracer degradation during incubation, we measured the ability of preincubated peptide to bind to fresh membranes as previously described (1). Briefly, <sup>125</sup>I-Tyr<sup>11</sup>-SS (250 pM) was incubated with membranes from rat frontoparietal cortex (0.15 mg protein/ml) for 60 min at 30°C. After this preincubation, aliquots of the medium were added to fresh membranes and incubated for an additional 60 min at 30°C. The fraction of the added radiolabelled peptide which was specifically bound during the second incubation was measured and expressed as a percentage of the binding that had been obtained in control experiments performed in the absence of membranes during the preincubation period.

#### Adenylyl Cyclase Assay

AC activity was measured as previously reported (31) with minor modifications. Briefly, rat frontoparietal cortical membranes (0.06 mg/ml) were incubated with 1.5 mM ATP, 5 mM MgSO<sub>4</sub>, 10  $\mu$ M GTP, an ATP-regenerating system (7.5 mg/ml creatin phos-

phate and 1 mg/ml creatine kinase), 1 mM IBMX, 0.1 mM PMSF, 1 mg/ml bacitracin, 1 mM EDTA, and test substances (10<sup>-4</sup> M SS or 10<sup>-5</sup> M FK) in 0.1 ml of 0.025 M triethanolamine/HCl buffer (pH 7.4). After a 15 min incubation at 30°C, the reaction was stopped by heating the mixture for 3 min. After cooling, 0.2 ml of an alumina slurry (0.75 g/ml in triethanolamine/HCl buffer, pH 7.4) was added and the suspension centrifuged. The supernatant was taken for assay of cyclic AMP by the method of Gilman (28). The concentration of SS used was that necessary to achieve inhibition of rat (55) and human (5,27) brain AC. FK was used at a concentration that could effectively stimulate the AC catalytic subunit of rat brain (55).

#### Data Analysis

The computer program LIGAND (39) was used to analyse the binding data. The use of this program enabled models of receptors that best fit the given sets of data to be selected. The same program was also used to present the data in the form of Scatchard plots (53) and to compute values for the receptor affinity (K<sub>d</sub>) and density (B<sub>max</sub>) that best fit the sets of binding data for each rat. Statistical comparisons of all the data were carried out with one way analysis of variance (ANOVA) and significant differences between experimental groups were evaluated by Student's Newman-Keuls multiple comparison procedures. Means among groups

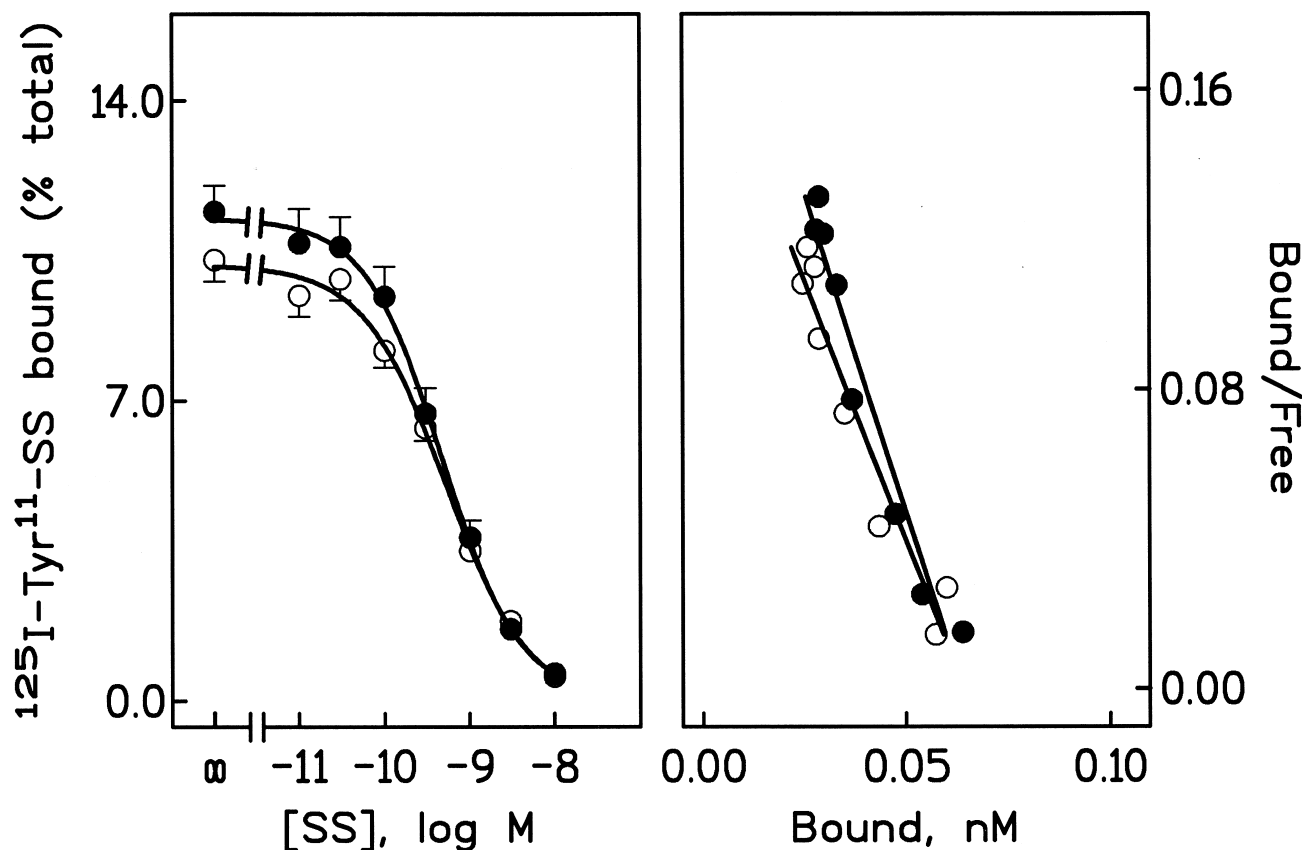


FIG. 2. Effect of mepyramine plus 2-pyridylethylamine (PEA) on somatostatin (SS) binding to rat frontoparietal cortical membranes. Mepyramine (30 mg/kg, IP) was administered 1 h before PEA (10  $\mu$ g/rat, i.c.v.). *Left panel:* Competitive inhibition of specific  $^{125}$ I-Tyr<sup>11</sup>-SS binding by unlabelled SS to frontoparietal cortical membranes. Membranes (0.15 mg protein/ml) were incubated for 60 min at 30°C in the presence of 250 pM  $^{125}$ I-Tyr<sup>11</sup>-SS and increasing concentrations of native peptide. Points correspond to values for the animals in the control group (●) and mepyramine plus PEA-treated group (○). Each point is the mean  $\pm$  SEM of five replicate experiments. *Right panel:* Scatchard analysis of the binding data.

were considered significantly different when the *P* value was less than 0.05. Each individual experiment was performed in duplicate.

#### RESULTS

The administration of PEA or mepyramine did not affect SSLI content in the rat frontoparietal cortex as compared with the control group (Table 1).

The specific binding of  $^{125}$ I-Tyr<sup>11</sup>-SS to frontoparietal cortical membranes was time-dependent in all the experimental groups; an apparent equilibrium was reached and maintained between 50–180 min at 30°C (data not shown). All subsequent binding experiments were therefore conducted at 30°C for 60 min. The specific binding of  $^{125}$ I-Tyr<sup>11</sup>-SS to rat frontoparietal cortical membranes was significantly increased in PEA-treated rats at a dose of 2.5, 5, 10 or 20  $\mu$ g/rat as compared to control conditions (Fig. 1, left panel). This increase is due to an increase in the maximal number of SS receptors and dissociation constant, as revealed by Scatchard plots of the binding data (Fig. 1, right panel; Table 1). Subsequent studies were carried out with the dose of 10  $\mu$ g/rat, the dose frequently used by other investigators (11). The addition of  $10^{-5}$  M PEA to the incubation medium changed neither the number nor the affinity of the SS receptors in the membranes of normal rats (data not shown). The stability of the radioligand in membranes from different groups was measured to examine the possibility that

decreased  $^{125}$ I-Tyr<sup>11</sup>-SS degradation might account for increased SS binding by these membranes. SS inactivation by plasma membranes after a 60 min incubation at 30°C was similar (8–10%) in all experimental groups. The administration of mepyramine at a dose of 5, 10, 30 or 60 mg/kg had no observable effect on SS binding. Subsequent studies were carried out with 10 mg/kg, the dose frequently used by other investigators (42). Pretreatment with the H<sub>1</sub>-receptor antagonist mepyramine abolished the PEA-induced changes in SS binding (Fig. 2, Table 1).

In a previous study carried out by our laboratory, inhibition of basal and FK-stimulated AC activity in frontoparietal cortical membranes from control rats was observed at a concentration of  $10^{-4}$  M SS, with no significant inhibition at lower doses (50). In the present study, additional experiments have been carried out to study the effect of a higher dose of SS on inhibition of AC activity in order to determine if  $10^{-4}$  M is a maximal concentration. In this regard, the SS-mediated inhibition of AC observed at  $10^{-3}$  M SS was the same as that observed at  $10^{-4}$  M. Therefore, all subsequent experiments on SS-mediated inhibition of AC activity were carried out with  $10^{-4}$  M SS. The functional coupling of the SS receptors to the AC system was studied in frontoparietal cortical membranes from control, PEA-, mepyramine- and mepyramine plus PEA-treated rats. No significant differences were seen for either the basal or FK-stimulated AC enzyme activities in any of



TABLE 2

EFFECT OF SOMATOSTATIN (SS) ( $10^{-4}$ M) AND FORSKOLIN (FK) ( $10^{-5}$  M) ON BRAIN ADENYLYL CYCLASE (AC) ACTIVITY (PMOL CAMP/MIN/MG PROTEIN) IN FRONTOPIRIETAL CORTEX MEMBRANES FROM CONTROL ( $n = 15$ ), 2-PYRIDYLETHYLAMINE (PEA) ( $n = 5$ ), MEPYRAMINE ( $n = 5$ )- AND MEPYRAMINE PLUS PEA- ( $n = 5$ )-TREATED RATS.

	PEA		Mepyramine + PEA		Mepyramine	
	Control	Treated	Control	Treated	Control	Treated
Basal activity	174 $\pm$ 8	154 $\pm$ 6	160 $\pm$ 4	153 $\pm$ 5	165 $\pm$ 4	159 $\pm$ 3
+ $10^{-4}$ M SS	130 $\pm$ 8	105 $\pm$ 4*	124 $\pm$ 3	114 $\pm$ 3	131 $\pm$ 5	121 $\pm$ 3
%SS inhibition of basal activity	25 $\pm$ 2	32 $\pm$ 1*	23 $\pm$ 2	25 $\pm$ 3	21 $\pm$ 3	24 $\pm$ 2
+ $10^{-5}$ M FK	1065 $\pm$ 44	1051 $\pm$ 24	956 $\pm$ 41	933 $\pm$ 35	921 $\pm$ 38	827 $\pm$ 10
Fold FK stimulation over basal	6.1 $\pm$ 0.2	6.8 $\pm$ 0.3	6.0 $\pm$ 0.3	6.1 $\pm$ 0.4	5.6 $\pm$ 0.2	5.2 $\pm$ 0.2
$10^{-5}$ M FK + $10^{-4}$ M SS	850 $\pm$ 59	695 $\pm$ 18*	740 $\pm$ 27	720 $\pm$ 26	658 $\pm$ 17	644 $\pm$ 31
%SS inhibition of FK stimulation	20 $\pm$ 2	34 $\pm$ 1***	22 $\pm$ 2	23 $\pm$ 1	26 $\pm$ 3	22 $\pm$ 4

Experiments were performed as described in Methods. Values represent the mean  $\pm$  S.E.M. of the determinations performed. Statistical comparison versus control: \* $p < 0.05$ , \*\*\* $p < 0.001$ .

the experimental groups (Table 2). In frontoparietal cortical membranes from all the experimental groups studied, SS ( $10^{-4}$  M) inhibited the basal and FK-stimulated AC activities (Table 2), which is in agreement with other authors (37,55). In the PEA group, however, the capacity of SS to inhibit basal and FK-stimulated AC activity in the frontoparietal cortex was significantly higher than in the control group (Table 2).

The stable GTP analogue 5'-guanylylimidodiphosphate [Gpp(NH)p] was employed to evaluate if there were changes in the functional activity of Gi proteins which might explain the greater SS-mediated inhibition of AC activity observed in the present study. Low concentrations of Gpp(NH)p inhibited FK ( $3 \times 10^{-6}$  M)-stimulated AC activity in frontoparietal cortical membranes from control and PEA-treated rats, with a maximum inhibition being observed at a concentration of  $10^{-7}$  M (Fig. 3). This inhibitory effect has been used by several authors as a measure of Gi functional activity (61,68). No significant differences in Gpp(NH)p-mediated inhibition of AC activity were observed between the control and PEA-treated animals (Fig. 3).

Pretreatment with the H<sub>1</sub>-receptor antagonist mepyramine prevented the PEA-induced changes in SS-mediated inhibition of AC activity (Table 2) whereas mepyramine alone had no observable effect on these parameters (Table 2).

#### DISCUSSION

This study is the first demonstration that a H<sub>1</sub>-histaminergic agonist induces an increase in the number of SS receptors, which is associated with an increase in SS-mediated inhibition of AC activity in the rat frontoparietal cortex.

No changes in SSLI content were detected after PEA or mepyramine administration. These results are in good agreement with a study of De los Frailes et al. (20) who observed that histamine caused no significant differences in either SS release or SS intracellular content in rat fetal cerebral cortical cells in culture.

H<sub>1</sub>-histaminergic receptors seem to mediate the action of PEA on the somatostatinergic system since the changes in the SS receptor/effector system induced by this agonist were prevented by pretreatment with the H<sub>1</sub>-receptor antagonist mepyramine. Moreover, the addition of PEA or mepyramine in vitro to frontoparietal cortical membranes obtained from untreated rats did not affect the SS binding parameters.

The SSLI content and equilibrium parameters of the SS receptors in the frontoparietal cortex of control rats were similar to those

previously reported by others (48,58). To date, five different SS receptor subtypes have been cloned (4,32), all of which appear to be expressed in the rat cerebral cortex, as demonstrated by a study of the tissue distribution of the messenger ribonucleic acid (7,32,47). The fact that this study with <sup>125</sup>I-Tyr<sup>11</sup>-SS shows linear Scatchard plots indicates that all the SS receptor subtypes have similar high affinity for the radioligand used.

The rapid changes in SS binding observed after PEA administration are in keeping with the modifications in this neuropeptide which were provoked by TRH (56) or cysteamine (59). In addition, Bruno et al. (8) observed a rapid increase in SS receptor mRNA as soon as 2 h following agonist treatment in GH<sub>3</sub> cells. Thus, the increase in SS binding observed after PEA administration may be due to synthesis of some SS receptors from existing or newly transcribed mRNA.

We previously demonstrated that an acute i.c.v. administration of histamine increases the SS receptor-effector system in the Wistar rat frontoparietal cortex (50). The present findings, therefore, suggest that the H<sub>1</sub>-histaminergic system is involved, at least in part, in this histamine-induced modulation of the somatostatinergic system.

It is now well established that histamine stimulates phosphoinositide breakdown in rat and guinea-pig brain slices through activation of H<sub>1</sub>-receptors (2,6,14,18). Therefore, it is tempting to speculate that phosphoinositide hydrolysis induced by H<sub>1</sub> agonists may cause changes in the overall conformation of the cell membrane which may induce the exposure of a pool of presynthesized SS receptors sequestered within the plasma membrane.

The data reported here show that frontoparietal cortical SS receptors are coupled in an inhibitory fashion to AC. The inhibition was already present in basal conditions, which is in agreement with previous reports (5,27,55). A relatively high concentration of SS was required to produce inhibition of AC, although the same concentration has been shown by other authors to be necessary for SS-mediated inhibition of rat (55) and human brain (5,27) AC activity. Several lines of evidence, however, indicate that the inhibition of AC activity achieved by  $10^{-4}$  M SS is not a nonspecific effect but rather is mediated by SS receptors. In this regard, the GTP dependence of the inhibitory effect suggests the involvement of a G protein in the response. This finding is consistent with binding studies on postmortem human and rat brain tissue which have shown that the binding of SS to its recognition site is affected

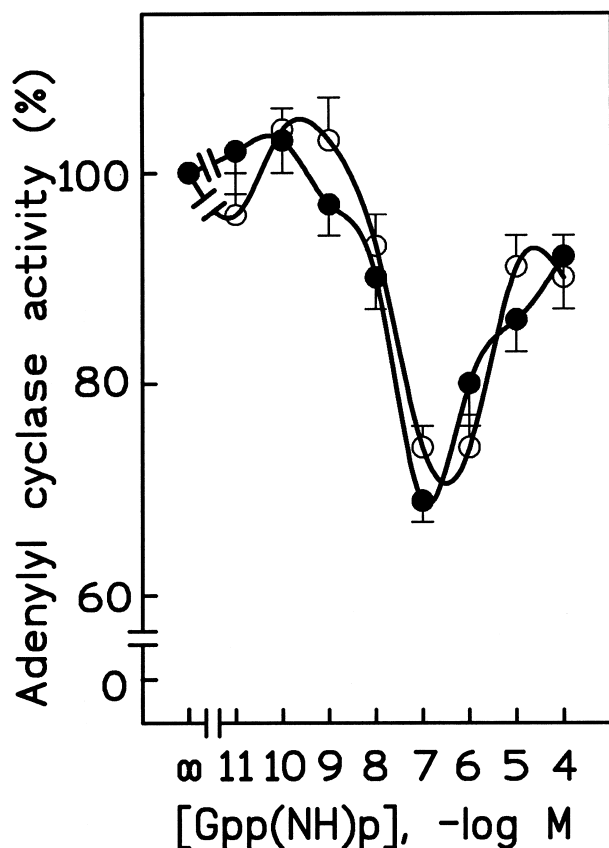


FIG. 3. Dose-effect curves for 5'-guanylylimidodiphosphate [Gpp(NH)p]-mediated inhibition of adenylyl cyclase (AC) activity in rat frontoparietal cortex membranes from control (●) and 2-pyridylethylamine (PEA)-treated (○) rats. Curves for the action of Gpp(NH)p on AC activity were carried out in the presence of  $3 \times 10^{-6}$  M forskolin (FK) and the indicated concentrations of Gpp(NH)p. Data are expressed as a percentage of FK-stimulated AC activity in the absence of Gpp(NH)p (100%). The results are given as the mean  $\pm$  SEM of five determinations performed in duplicate. No statistically significant differences were obtained between control and PEA-treated rats.

by GTP in a manner consistent with the involvement of a G protein (27).

It has been shown that SS inhibits basal AC activity in neuronal but not glial cells, and in membranes from the cerebral cortex, striatum and mesencephalic regions of mouse embryos (12,13,15,66). In a fresh astrocyte-rich suspension from the rat cerebral cortex, vasoactive intestinal peptide (VIP), an agonist acting through Gs, was observed to stimulate cAMP accumulation up to 2.3 times the basal levels whereas SS did not inhibit this

effect at any of the VIP concentrations (15). Due to the differential response of neuronal and glial cells to AC inhibition by SS, it is possible that the cell heterogeneity of the present preparations may be the cause of the high SS concentration necessary to obtain inhibition of AC, both by our group and by other authors. In addition, the synaptic concentration of SS is, to date, unknown. On the other hand, glial SS receptors have a lower affinity for the peptide than neurons (15).

The ability of SS to inhibit basal and FK-stimulated AC was increased in frontoparietal cortical membranes from PEA-treated rats as compared with the control values. No significant differences were observed, however, between the PEA-treated and control groups for either basal or FK-stimulated AC activity in the frontoparietal cortex. This result suggests that the integrity of at least the catalytic subunit of the enzyme is intact in the PEA-treated group.

The functional activity of Gi proteins was measured in control and PEA-treated rats in order to determine if the greater inhibition of AC by SS observed in the PEA group was due to a change at this level. In both control and PEA-treated rats, the nonhydrolyzable GTP analogue Gpp(NH)p elicited a characteristic biphasic effect on FK-stimulated AC activity, with low concentrations inhibiting FK-stimulated AC activity and high concentrations stimulating it. This biphasic effect is due to the activation of Gi and Gs proteins, respectively, due to the higher affinity of Gi proteins for Gpp(NH)p (61). The lack of changes in the inhibitory effect of Gpp(NH)p on FK-stimulated AC activity in cortical membranes from PEA-treated rats suggests that the increased SS inhibition of AC activity is not a result of an increase in Gi protein-AC coupling but is most likely related to the observed increase in the number of SS receptors.

The significance of these results is, at present, difficult to assess. Since a significant decrease in histamine content (38) and SS content and receptors (3) has been described in certain neuropsychiatric disorders such as Alzheimer's disease, the present findings could be of interest in understanding the pathophysiology of such disorders.

In summary, the present study reveals the existence of *in vivo* interactions between the  $H_1$ -histaminergic and the somatostatiner-gic systems in the rat frontoparietal cortex. Since SS and histamine modulate higher nervous activity (19,26,43), behavioral arousal (17,33,64) and seizure susceptibility (35,48,54) in a similar manner, the increase in the number of SS receptors may reflect a positive feedback mechanism further enhancing the modulatory effects of SS on histaminergic neurotransmission. Since the  $H_1$  antagonist mepyramine had no effect on the measured parameters, it is tempting to speculate that endogenous histamine might have a phasic influence on SS receptors rather than a tonic effect.

#### ACKNOWLEDGEMENTS

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